# A STUDY OF THE PROBLEM OF WATER ABSORPTION.

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## I. INTRODUCTION.

When we observe under the microscope a piece of plant tissue, an algal filament, or the spores of a fungus, in that condition that we are pleased to call normal, we find that in each case the cells are quite rigid. This rigidity, or turgidity, as it is generally termed, is due to the fact that the protoplast exerts a certain amount of pressure on its cell wall. When a cell becomes plasmolysed the protoplasm ceases to exert any pressure on the cell wall, and we say that such a cell has lost its turgor.

It is a difficult problem to explain the origin of the force from which results turgidity in living cells. The explanation most generally given is based on the assumption that a semi-permeable membrane or, at least, a membrane possessing only limited permeability for dissolved substances surrounds each cell. When such a membrane is interposed between two solutions of different concentrations, the major current of the solvent is toward the more concentrated solution, and osmotic pressure becomes manifest. In this way is explained the entrance of water into cells. If we assume that a semi-permeable membrane surrounds the living cell, we may account for the pressure observed in turgid cells by saying that it is due to osmotic pressure resulting from a difference in concentration between the solutions inside and outside of the cell. The purpose of this paper is to give the results of some experiments that bear upon this problem and to consider to what extent we are justified in assuming the existence of a semi-permeable living membrane.

This assumption that the cell is an osmotic system carries with it certain conclusions, for osmotic pressure is known to

obey certain laws. When the temperature is kept constant the osmotic pressure of a given solution depends only on the number of dissolved particles in that solution. The chemical nature of these particles has no effect on osmotic pressure; they may be the ions or the molecules of any chemical substance; in other words, osmotic pressure obeys the gas laws. That this simple explanation of turgor and of its cause, the entrance into and retention of water by living cells, is inadequate, becomes evident when one makes a study of the literature dealing with our problem.

## II. LITERATURE.

It was long ago observed by Loeb (9) that the amount of water absorbed by muscle tissue when placed in equimolecular solutions of various electrolytes depends on the nature of the dissolved substance. In a certain solution of potassium chloride, for example, the muscle tissue was found to gain from forty to fifty per cent of its original weight after eighteen hours, while in an equimolecular solution of sodium chloride it gained only seven per cent of its original weight. We see that the osmotic theory of the passage of water into cells is not in accord with these observations.

In order to explain such facts and at the same time keep the osmotic theory of water absorption, it has been necessary to suppose that the permeability of the living membrane varies with external conditions. Fernbach (3) showed that dilute alcohol makes the plasma membrane of the yeast cell permeable to invertase. Harvey (6) found that neutral red and other basic dyes fail to enter cells in the presence of a very weak acid. He also found that weak alkalies enter cells, while strong alkalies do not, thus showing that permeability varies with alkalinity. Lavison (7) found that the protoplasm of peas and lupines is permeable to the salts of heavy metals when they are offered in high concentrations, while these same salts do not enter the cells from solutions of low concentration. According to Paine (14) yeast cells take up sodium chloride, ammonium chloride, copper sulphate, and sodium phosphate from moderately concentrated solutions,

but can take up only sodium chloride and ammonium sulphate from dilute solutions. Fluri (5) claims that aluminum salts render the protoplasm of *Spirogyra* cells permeable to ordinary plasmolytic agents. Wächter (18) found that sugar diffuses out of sections of onion in distilled water or in hypotonic sugar solutions, but that the addition of a trace of a potassium salt to the solution prohibits the diffusion of the sugar from the onion tissue. Osterhout (12) has concluded that the antagonistic action of various salts as regards their toxic effects is largely or entirely due to the fact that they hinder the entrance of each other into the protoplasm, *i. e.,* that each salt affects the permeability of the protoplasm for other salts. True and Bartlett (17) have shown that the rate of absorption and secretion by the roots of peas varies with the concentration of the solution in which the roots are immersed and with the nature of the salt or salts dissolved in the solution. McClendon (10) thinks that those substances that stimulate growth do so by affecting the permeability of the protoplasm of the cells stimulated. Czapek (2) found that tannin diffuses from cells of *Echeveria* whenever the surface tension of the medium in which the cells are immersed is lowered to approximately two-thirds that of water. It is claimed by Ramann and Bauer (16) that the time of maximum absorption of an element by the roots of trees varies with the season. The pine, for example, absorbs nitrogen most rapidly in June, while it takes up calcium most rapidly in August.

These observations, made by a number of different workers, show that the membrane, which has been assumed to surround living cells, must, if it exists, alter its permeability under many different conditions. The permeability of non-living membranes is not capable of such alterations. The importance of the supposed membrane surrounding living cells depends entirely, so far as absorption and secretion are concerned, on its degree of permeability. Since the living membrane differs from the non-living membranes in this important characteristic, it naturally follows that we should be very careful when we compare the one with the other. The

effect of a given solution on the turgor of living cells cannot be predicted from a knowledge of the osmotic pressure of that solution. The nature of the dissolved substance is quite important when we are dealing with living cells.

Many facts at variance with the osmotic theory of water absorption have been explained by saying that the living membrane is not quite semi-permeable and that its permeability is different at different times and under different circumstances, but there are certain phenomena that are not to be explained in this simple manner. When the small intestine of a cat was partially filled with a certain watery solution, Cohnheim found that there was a passage of water out of the gut. He explained irreciprocal permeability by saying that two forces are here concerned; one the force of osmotic pressure and the other a force independent of osmotic pressure and located in the living cells.

The secretion from the kidneys contains some substances in higher and some in lower concentration than the blood; it contains less chlorides and more sulphates and urea than does the blood (4). We may explain the passage of urea from the blood to the urine, until its concentration in the latter is equal to that in the former, as due to diffusion, but it is not possible to explain how it becomes more concentrated in the urine than in the blood in this way. Blood corpuscles that take their nourishment from the serum in which they are immersed are rich in potassium phosphate, while the serum is poor in this substance, but rich in sodium chloride (15). Selective absorption has been explained by stating that the substances absorbed are those to which the membrane is permeable, while those that are not absorbed are unable to pass through the living septum. This explanation is very satisfactory until we come to those cases where the final concentration of the diffusing substance becomes greater inside than outside of the cell. This has been explained by supposing that some of the substance, after passing into the cell, enters into combination with some other substance and is thus rendered incapable of diffusion. But there is little evidence to show that this actually takes place. Besides, it has been

found that in certain cases the diffusing substance is less concentrated inside than outside of the cell, although it readily passes into the protoplasm. Paine (14) found that on immersing yeast cells in alcoholic solutions varying in concentration from five to twenty per cent, the ratio of the concentration of the alcohol within the cell to that of the solution outside was practically a constant, and independent of the absolute concentration. The alcohol outside of the cell was always more concentrated than the alcohol inside of the cell. Paine explained this observation by supposing that some of the water within the cell is combined in such a way that it is unable to act as a solvent for alcohol.

The osmotic theory fails us most completely, however, when we come to cases of permanent plasmolysis of cells by substances that readily enter the protoplasm. Lavison (7) found that sodium chloride passes into the protoplasm even from plasmolysing concentrations and causes permanent plasmolysis, although large quantities of the salt are taken up by the protoplasm. He further found that when he plasmolysed cells with an iron salt, the salt, nevertheless, entered the protoplasm, and that if the experiment was not continued too long the cells were not killed by such treatment. How is it possible for a salt that readily passes through the membrane to cause plasmolysis? So far as such salts are concerned the membrane does not exist, and yet we observe a phenomenon (plasmolysis) that is ordinarily explained as due to the action of a semi-permeable membrane.

It must be obvious from the foregoing enumeration of facts recorded and conclusions reached by various workers that diffusion and osmosis do not explain absorption and secretion. There is great confusion regarding the nature of the membrane that has been assumed to surround cells. Lepeschkin (8) has considered it a protein membrane, Overton (13) a lipoid membrane, Nathansohn (11) a membrane in which lipoid particles are mixed with protein particles, while Czapek (2) looks on it as an emulsion of fat, each globule of which is surrounded by a thin layer of soap. All of these things cause us to doubt the value of the assumption that a semi-

permeable membrane or a membrane that is only partially permeable to dissolved substances surrounds living cells.

The literature shows that there are numerous facts that do not accord with the assumption that such a membrane regulates absorption and secretion. But it is certainly not possible to prove that a membrane possessing some degree of semi-permeability does not surround certain cells. The best we can do is to determine the value of the assumption that such a membrane does exist. It was with these facts in mind that a study of this problem was undertaken.

## III. EXPERIMENTAL STUDY.

### 1. Mechanical Injury to the Membrane.

Since the permeability of living protoplasm is quite susceptible to change, and since its permeability is supposed to result from the character of a living membrane surrounding the protoplast, one must naturally expect this membrane to be easily capable of mechanical injury. The following observations bear on this phase of our problem:

When one places vigorous filaments of *Spirogyra setiformis* (Roth) Kütz., which have been growing in pond water into a strong solution (solutions about two times molar were used) of potassium or sodium chloride and observes them with a low-power microscope, the cells do not appear to be plasmolysed, but when they are observed more closely and stronger magnification is used one sees that they are very much plasmolysed. The protoplast now occupies only a small fraction of its original volume and forms one or more spherical masses within the cavity surrounded by the chlorophyll bands. If the cells are carefully observed during the course of this experiment it will be seen that when the strong chloride solution surrounds them they become plasmolysed very quickly. The chlorophyll bands often occupy exactly the same position after plasmolysis that they occupied when the cell was in its normal condition. If a weaker solution is used, plasmolysis takes place more slowly and the chlorophyll bands are displaced toward the center of

the cell by the contracting protoplasm. When this happens the bands generally lose their spiral form and appear as irregular masses of green.

Thus, when the cells are plasmolysed by a strong solution, the chlorophyll bands are not displaced and lie, after plasmolysis, entirely outside of the protoplasm. If, on the other hand, a weaker solution is used and plasmolysis is brought about more slowly, the chlorophyll bands are carried toward the center of the cell and lie, after plasmolysis, entirely within the protoplasmic mass. When the cells plasmolysed by a strong solution are observed under a low-power microscope, they do not appear to be plasmolysed. The chlorophyll bands still occupy their normal position, and the spherical protoplasmic masses being quite hyaline are difficult to distinguish.

When the cells are plasmolysed by a strong solution, the membrane surrounding the protoplasm must be broken in many places in order to allow the escape of the chlorophyll bands. If the membrane is differentiated from the remainder of the protoplasm, *i. e.,* if there is really a membrane surrounding the protoplast, one would expect it to lose its semipermeability after such treatment. Certainly no inert membrane with which we are acquainted could be used to demonstrate osmosis after being broken in so many places. The spherical masses of protoplasm that have been freed of their spiral bands will, nevertheless, take up water and enlarge if the solution in which they are immersed be diluted with distilled water. No success has accompanied efforts to make the spherical masses enlarge sufficiently to fill the entire cell. They always burst and disintegrate before they have enlarged to that extent. These protoplasmic masses take up water, although any membrane which may have surrounded the cell in its normal condition must have been broken when the chlorophyll bands passed out through the surface layers of the protoplasm. This experiment was often repeated, and always with the same results.

If a membrane surrounds these *Spirogyra* cells in their

normal condition, then, either the integrity of that membrane is not lost when it is broken or a new membrane is formed about the protoplasm very soon after the old one is destroyed. The spherical masses within the plasmolysed cells show themselves capable of taking up water, even when the solution is diluted immediately after plasmolysis. It is hard to think that the short period of time necessary to dilute the plasmolysing solution is sufficient to allow of much differentiation in the outer layers of the protoplasm. If we dispense with the idea that a new membrane is formed immediately after the old one is disrupted, then the only conclusion to which we can come is that the membrane surrounding the normal protoplast, if such a membrane exists, is not very susceptible to mechanical injury. One would expect to find a living membrane more delicate than non-living membranes, yet we do not know of an inert membrane through which solid bodies may be forced without rendering it quite permeable.

### 2. The Concentration of Cell-Sap.

Along with the assumption that a semi-permeable membrane surrounds the living plant cell, goes the further assumption that the solution contained within the membrane is as concentrated if not more concentrated than the liquids surrounding the turgid cell. If this were not true the cell would not remain turgid, but would lose some of its water and become plasmolysed. If, therefore, cell-sap be obtained from cells that are as nearly alike as possible it should represent the concentration of the solution originally within the cells. If cells like those used in this experiment be placed in the sap, they should remain practically normal as regards their turgor. But if this sap solution be concentrated it should then plasmolyse these cells. In order to determine whether or not this would take place, the following experiment was performed:

*Monilia sitophila* (Mont.) Sacc. was grown on pieces of potato that had been washed in running tap water for forty-eight hours. From these cultures fifty-two and one-half grams of spores were collected. They were obtained by inverting the cultures and shaking them above a piece of paper.

They were harvested three weeks after the cultures were started. Spores were taken only from pure cultures of the fungus. They were ground up in a mortar with fine quartz sand. Before being used this sand was boiled in alkali, then in acid, and finally in several changes of distilled water, in order to free it of impurities. The entire mass of spores after being ground up in this way, was subjected to a high pressure, and several cubic centimeters of sap were squeezed out. This sap solution was reddish-yellow in color, had a specific gravity of about 1.053, contained absolutely no reducing sugars, was neutral to litmus and gave, on the addition of alcohol or when heated above 50° C., a heavy floculent precipitate.

Some spores and mycelial cells exactly like those used for obtaining sap were placed in this solution. After sixteen hours none of the cells showed plasmolysis; most of them had germinated and the solution was filled with mycelium. Cells of *Spirogyra setiformis,* on the other hand, became quickly plasmolysed when placed in the sap of the *Monilia* spores. Some of the sap solution was boiled down to one-tenth of its original volume. In this concentrated solution the spores and mycelial cells became quickly and permanently plasmolysed. The exact concentration of the sap that would be just sufficient to plasmolyse the cells was not determined, but it was found that even after considerable concentration the sap solution would not cause plasmolysis. This result indicates that the *Monilia* cells do not behave as ordinary osmotic systems; if they did we should expect them to become plasmolysed in solutions of their own sap, even when such solutions are only slightly more concentrated than the sap. In slightly concentrated solutions of their cell-sap the spores germinate and grow. From this it seems that spores of *Monilia* are able to take up water from solutions more concentrated than their own cell-sap.

As was mentioned above, the sap solution was of a reddish yellow color. It is interesting to note in this connection that when the spores are placed in the sap solution they readily take up the coloring matter and become a much deeper color

than the sap itself. Even in the sap solution concentrated to one-tenth of its original volume, the spores, though they became plasmolysed, absorbed so much of the coloring matter that they appeared quite yellow when observed under the microscope. They became a much deeper color than the solution that surrounded them. This is an interesting case of selective absorption, and one in which the substance absorbed is a product of the cells that show the absorption. Moreover, the coloring matter becomes more concentrated inside than outside of the cell. This, like other examples of the same phenomenon, cannot be explained as due to diffusion. Diffusion would tend to equalize the concentration of the coloring matter inside and outside of the cell. If a mass of *Monilia* cells are placed in a small amount of water and left for several hours, the water does not become colored. None of the coloring matter diffuses out of the cells. When, however, the cells are placed in a solution of *Monilia* cell-sap they quickly take up the coloring matter and become a much deeper color than before. Thus it seems that the reddish yellow substance contained in *Monilia* cells that are grown in the light is capable of passing into, but not out of, these cells. A semi-permeable membrane does not help us to explain this observation.

### 3. Absorption of Water by Egg-Albumen.

If a small amount of egg-albumen be placed in a paper bag and suspended in distilled water, the albumen will take up water and the bag will gain in weight. Much less weight is gained when such a bag is suspended in a strong solution of sodium or potassium chloride. A number of experiments have been performed for the purpose of determining the effect of different concentrations of sodium chloride on the absorption of water by egg-albumen. In all of these experiments the egg-albumen was taken from fresh hen's eggs, placed in small paper bags and immersed in the solution to be tested.

After bag No. 1 had remained in a 2M sodium chloride solution for eighteen hours and had gained only 1.2 per cent of its original weight, as shown by the table below, it was re-

moved from the sodium chloride solution and suspended for 20 hours in distilled water. At the end of this period of time it weighed 41.5 grams, having gained more than 10 grams during the 20 hours in distilled water. Bag No. 4, which, as shown by the following table, had gained 44 per cent of its original weight during the 18 hours that it was suspended in distilled water, was, at the end of this period of time, removed from the distilled water and placed for 20 hours in 2M sodium chloride solution. At the end of 20 hours it weighed 37.4 grams, having lost 1.7 grams during the 20 hours that it was suspended in the 2M sodium chloride solution. At the end of the experiment No. 1 had gained 43 per cent of its original weight, while No. 4 had gained only 36 per cent of its original weight. During the 20 hours in distilled water, No. 1 gained 41.8 per cent in weight, while during the same period of time No. 4, in 2M sodium chloride solution, lost 5.3 per cent in weight.

The following table shows the results of such an experiment:

| Number on bag | Weight of bag | Weight of bag plus albumen | Concentration of solution used | Weight of bag after 18 hours in solution | Per cent gain in Weight |
|---|---|---|---|---|---|
| 1 | 6.9 gr. | 31.0 gr. | 2 M NaCl | 31.3 | 1.2 |
| 2 | 7.0 " | 26.9 " | M NaCl | 29.7 | 14.0 |
| 3 | 7.1 " | 30.6 " | $\frac{M}{2}$ NaCl | 34.8 | 18.0 |
| 4 | 7.1 " | 29.3 " | Distilled water | 39.1 | 44.1 |

The egg-albumen used in this experiment is certainly quite different from living protoplasm, and yet it is the substance that we ordinarily think of as being most like protoplasm. The white of the egg is a colloidal solution of albumens. As Lavison (7) has pointed out, protoplasm is principally a colloidal solution of albumens. The paper used to separate the egg-albumen from its surrounding medium in the above experiment differs in some respects from the cellulose wall that surrounds ordinary plant cells. Nevertheless, the two are much alike. The paper differs from the cell wall in physical structure, but the two are quite similar chemically. The paper, like the cell wall, is permeable to both water and electrolytes.

While this artificial cell differs from the living plant cell, the two are alike in several ways. The artificial cell takes up water from a dilute solution, but gives up water when transferred from a dilute to a concentrated solution. The living cell takes up water from dilute solutions, but gives up water when placed in concentrated solutions. The layer of egg-albumen in contact with the paper bag certainly does not become differentiated so as to form a semi-permeable membrane, for egg-white is miscible with water in all proportions.

Osmotic pressure is sometimes supposed to be demonstrated by breaking some of the shell from one end of an ordinary hen's egg and then placing it in a tumbler of water. After a period of time the membrane surrounding the egg bulges at the point where the shell was removed, thus showing that a pressure has developed inside of the membrane. The membrane surrounding the egg may not be permeable to certain dissolved substances, but a paper bag that is quite permeable to these will serve just as well as the membrane when we wish to show that egg-albumen will take up water from dilute solutions. The pressure developed within the egg when it is placed in a tumbler of water is, for the most part, I believe, not a result of osmotic pressure, but a pressure due to the affinity that the colloids in the egg have for the water outside of the egg. Sometimes, in experiments like this, so much water is taken up that the membrane becomes greatly distended and finally breaks. At this we need not be surprised. Fisher has shown that certain colloids have a strong affinity for water.[1]

## IV. DISCUSSION.

That diffusion enters into the problems of absorption and secretion by living cells cannot be doubted, but that such cells maintain their turgor by virtue of osmotic pressure and are surrounded by semi-permeable membranes is pure assumption. We can explain turgor and water absorption without assuming the existence of a living membrane possessing the

[1] Fisher has shown that the colloids in the eye of the sheep are capable of taking up water from certain solutions until so much pressure is developed that they burst the strong coat surrounding the eye.

property of semi-permeability (4). Moreover, such an explanation seems to accord better with facts than does the explanation based on the assumption that such a membrane exists. On the whole, the pressure developed in living cells does not obey the gas laws. In order to keep the osmotic theory of water absorption it is necessary to make various assumptions regarding the changes in the permeability of the assumed membrane. When we assume that a semi-permeable membrane exists, and undertake to become familiar with its properties, we find that they are not known. The permeability of the same cell seems to be affected differently by different substances. It changes as the concentration of the medium changes and seems to vary, even with the season of the year. The semi-permeable living membrane is an assumption, upon which is based a theory, which, it seems to me, is not only no longer useful, but even detrimental to a correct understanding of the phenomena of absorption and secretion. As Martin H. Fisher (4) has pointed out, we know very little regarding the affinity between colloids and watery solutions. We use the word "affinity" to cover our ignorance, but it seems better to do this than to make an assumption that does not find justification in the facts that are known.

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